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EXPRESSION OF FUNCTIONAL NMDA RECEPTORS IN HUMAN OA CHONDROCYTES

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Purpose: Classical neuronal signalling molecules such as substance P and glutamate have been identified in cartilage and are being shown to have roles in regulation of chondrocyte function. This study looks at the expression and function of the metabotropic glutamate NMDA receptor (NMDAR) in OA chondrocytes.
Methods: Chondrocytes from articular cartilage of human knee joint arthroplasty specimens. NMDAR subunit expression at gene and protein levels was identified by RTPCR and western blotting. NMDAR function was assayed using radolabelled Ca^{45} uptake and changes in cell membrane potential in response to NMDA and mechanical stimulation (MS; electrophys. only) in the presence and absence of the NMDA antagonists MK801 (non-competitive), Ifenprodil (NR2B selective) and APV (competitive).
Results: NMDAR1, 2A, and 2B, but not NR2C, 2D and 3, were detected at varying levels in OA chondrocytes at both the protein and RNA levels. Assessment of receptor function showed that OA chondrocytes responded to NMDA; this response was decreased by specific antagonists. NMDA induced an increase in Ca^{45} uptake which was reduced to baseline by the antagonists. Both NMDA and mechanical stimulation induced a membrane depolarisation which was decreased or abolished by NMDAR antagonists.
Conclusions: This study shows the presence of functional NMDA receptors composed of NR1, 2A and 2B subunits on human OA chondrocytes. It has been suggested that the NMDA receptor is a mechanosensitive receptor in neurones and the current investigation suggests similar involvement in chondrocyte mechanotransduction.

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RESPONSE OF CHONDROCYTES TO INJURY IN VIVO

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Purpose: Secondary osteoarthritis (OA) commonly occurs following joint injury. We developed a model system to examine the sequence of events that leads to osteoarthritis subsequent to traumatic injury. The goal of the current study was to determine the effect of a single blunt impact on cartilage in vivo in young rabbits. While most studies on cartilage impact have used explants of cartilage, our study is one of the first to examine the effects of traumatic injury in vivo.
Methods: 54 New Zealand white rabbits 3 months of age were subjected to blunt injury equal to 70% and 90% of the cartilage fracture threshold (Borrelli et al., 2002). Cartilage was harvested at 0, 1 and 6 months post-injury. Outcome measures were histology to detect proteoglycan and DNA, immunohistochemistry to determine the presence of proteins, in situ hybridization to mRNA to measure cellular matrix synthesis, and TUNEL to detect DNA fragmentation.
Results: At six months, all animals showed visual signs of OA including degenerated cartilage: no osteophyte was observed. At 1 month after injury, cells in the impact area lost BMP-2 staining, while cells and matrix surrounding the impact zone were positive for BMP-2. To determine whether these cells were actively synthesizing type II collagen, mRNA was assessed by in situ hybridization. At 1 and 6 months, only cells actively synthesizing BMP-2 were positive for type II procollagen mRNA. To determine the fate of these cells, tissue was stained with hematoxylin and eosin to detect nuclei and TUNEL to detect DNA breakdown. At 1 month, cells in the impact zone were intact with intact nuclei, and, although most cells had intact DNA, some TUNEL staining was observed in the upper 1/3 of the cartilage. At 6 months, cells in the lower half of the impact zone lost their nuclei and about 30% of the cells in the lower half were positive for TUNEL staining. Extensive cartilage degradation and proteoglycan loss was observed in the impacted regions where cells were no longer present. No significant difference was observed between the 60% and 90% impact levels. These results indicate that chondrocytes from young animals are surrounded by BMP-2-containing extracellular matrix and actively synthesize matrix molecules. After a single impact, cells in the impact zone loose their ability to synthesize matrix potentially due to the loss of signaling by BMP-2. Subsequently, cells undergo DNA fragmentation and are lost.
Conclusions: The results from this study indicate that post-traumatic arthritis may be different from primary arthritis. Hallmarks of human primary OA are the presence of osteophyte, specific enzyme-induced matrix degradation, and a high degree of anabolic activity. The occurrence of OA is thought to be due to the response of chondrocytes to the injury with active degradation of the collagen and proteoglycan of the extracellular matrix leading to cartilage degeneration. The results from this study demonstrate that young cartilage with actively synthesizing cells, is susceptible to degeneration following severe injury. Importantly, rather than attempting to repair the damage, the cellular response is passive and occurs in a stepwise manner: first, cells lose their capacity to synthesize matrix, then die, then the matrix degenerates. The lack of the formation of osteophytes is consistent with this passive degeneration. These studies open up an entirely new avenue for potential treatments of cartilage injury following trauma in that renewal of the biosynthetic capacity may be targeted.

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CYTOPROTECTIVE AGENTS (IGF-1 AND JNK-II) EFFECT ON ARTICULAR CARTILAGE UNDERGOING MECHANICAL CHONDROPLASTY

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Purpose: Partial thickness articular cartilage lesions are a common pathology in sports medicine and usually are a consequence of trauma from a sports injury. Mechanical chondroplasty is one technique used to smooth damaged articular cartilage but it may also induce metabolic damage to the surrounding cartilage. Insulin-like growth factor (IgF-1) and c-Jun N-terminal kinase inhibitor (JNK-II) may provide cellular support to the surrounding cartilage and enhance metabolic activity, if administered before or after chondroplasty.
Methods: Eight young bovine knees were obtained and divided into two groups. Group 1; knee condyle quarters were immersed in media containing either IgF-1 at 25, 50 or 100ng/ml or JNK-II at 10, 25, 50uM and incubated at 37°C for 24 hours. After 24 hours, chondroplasty was performed on the condyle quarters. Explants were cut (20-30 mg) and placed in 1mL media without IgF-1/JNK-II and incubated at 37°C until assayed. Proteoglycan synthesis (PG) was measured on Day 2, 4 and 8 using a dimethyl-methylene blue (DMMB) assay. Group 2; chondroplasty was performed immediately and explants were cut and placed in media containing either IgF-1 at 10, 25 or 50ng/ml or JNK-II at 5, 10 or 25uM. PG was measured on Day 1, 3 and 7.

Controls with and without chondroplasty were prepared for both groups. Media was changed every two days and group 2 media was supplemented with either IgF-1 or JNK-II. Eight 1mm slices from representative condyles were cut with a precision saw. Fours slice each were used for the cell viability and depth of penetration assay.
Results: Group 1: IgF-1 (50ng/ml) showed a significant improvement (p=0.03) in PG on Day 2. JNK-II (25uM) displayed improved PG without significance (p=0.06) on Day 2.
Group 2: IgF-1 (25ng/ml) significantly enhanced PG on Day 3 (p=0.03). IgF-1 (50ng/ml) significantly inhibited PG on Day 1, 3 and 7 while JNK-II (25uM) significantly inhibited PG on Day 1. The average depth of penetration for the representative cartilages slice was 99um while the depth of cell death averaged 151um.
Conclusions: The data suggest that a higher IgF-1 concentration is required to elicit the cytoprotective effect if administered prior to chondroplasty and a lower concentration must be used if IgF-1 is administered after chondroplasty. At higher concentrations (post-chondroplasty), IgF-1 and JNK-II inhibited proteoglycan synthesis. This suggests that a threshold concentration is needed to elicit the cytoprotective effect but higher concentrations inhibit PG. The mechanism of how these agents work in protecting cells needs further elucidation. This was an in vitro study which contains inherent weakness of not having synovial factors in the mileu. Bovine cartilage was used in this experiment instead of human osteoarthritic or cadaver cartilage due to availability and tissue quality. Human osteoarthritic chondrocytes have the potential to react differently to the cytoprotective agents and thus must be evaluated. Young bovine cartilage was used since chondrocytes proliferate much faster in young animals and may also have a greater response to cytoprotective agent than older tissue. The superficial zone of chondrocytes (within 100um of the articular surface) has the highest level of cell proliferation. Since the depth of penetration in our experiment averaged 99um, most active chondrocytes have been removed by chondroplasty. These data support the hypothesis that pre or post treatment of articular cartilage with IgF-1 increases metabolic activity and provides cytoprotection when performing chondroplasty under these conditions.

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MOUSE CARTILAGE UNDER COMPRESSION: INDUCTION OF NUCLEAR FACTOR-kB AND EXTRACELLULAR SIGNAL REGULATED KINASE1/2 ACTIVITY AND MODULATION BY AVOCADO/SOYBEAN UNSAPONIFIABLES

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Purpose: We studied the main intracellular signalling pathways known to be involved in the prodegradative process of matrix cartilage (MAP kinases and NF-κB) in chondrocytes stimulated with the proinflammatory cytokine interleukin-1 beta (IL-1β) or in cartilage explants submitted to a mechanical stress (1MPa, 0,5 Hz). Moreover, we studied whether Avocado-Soybean Unsaponifiables (ASU), a common drug used in Europe for symptoms in osteoarthritis (OA), could modulate these intracellular signalling pathways.
Methods: Mouse costal chondrocytes in monolayer primary culture stimulated with IL-1β (10ng/ml) or mouse costal cartilage explants under mechanical stress (MS) were used in this study. The chondrocytes or explants were incubated in presence or absence of ASU (10μg/ml) NF-κB pathway was assessed by IκBα expression, by nuclear translocation of NF-κB using p65 antibody, by Electrophoretic Mobility Shift Assay (EMSA), using p50 and p65 antibodies. MAP kinase (MAPK) pathways were assessed by using phospho-p38, ERK1/2 and SAP/JNK protein expression.
Results: I-kBα expression is decreased by 70% in compressed cartilage (after 2 hours of compression). I-kBα expression is also decreased by 72% in presence of IL-1β (as soon as 2 minutes after stimulation), in parallel with the translocation of the cytosolic p65 subunit to the nucleus. Moreover, the binding of the heterodimer p50/p65 to NF-κB responsive element is significantly increased after IL-1β treatment. Interestingly, ASU partially prevent ILβ-induced degradation of IκBα by 39% and MS-induced degradation of IκBα by 28%.
IL-1β-induced binding of p50/p65 is significantly inhibited in presence of ASU, in parallel with an inhibition of the translocation of p65 into the nucleus. Whereas the 3 MAPK p38, JNK and ERK1/2 were activated in presence of IL-1β, ASU inhibited specifically the ERK1/2 pathway by 34%. A same profile was observed in MS-activated chondrocytes.
Conclusions: We show here that, along with IL-1β, MS is also a strong trigger for NFκB and ERK 1/2 activation suggesting that these 2 pathways are mechanosensitive in chondrocytes. Moreover, our study shows that ASU inhibit NF-κB and ERK1/2 pathways.

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THERMOGRAVIMETRIC INVESTIGATION OF NORMAL AND DAMAGED HUMAN HYALINE CARTILAGE

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Purpose: The purpose of this study was to elucidated the importance of water content in contributing to disease progression and to establish the kinetic character of water loss effect of heating. Previously, water content has not been measured thermoanalyticaly in normal and degenerative human hyaline cartilage. Therefore a new thermogravimetric protocol had to be established before the detailed investigation could be performed. Most of the known changes in the extra cellular matrix in OA comes from animal models since human samples for investigation are not widely available for experiment. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. Thermogravimetry (TGA) is one of the oldest thermal analytical procedures and has been used extensively in the study of polymeric systems.
Methods: During arthroplasty procedures performed at the Orthopaedic Department, University of Szeged, Hungary, degenerative human hyaline cartilage was obtained from 28 hip and normal cartilage from 7 knee. The samples were taken under sterile conditions, and excess bone was removed. Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs and radiological findings. The state of the hyaline cartilage was determined intraoperatively. 35 samples were collected. Based on the patient diagnosis, seven samples were analyzed as normal hyaline cartilage, 12 were obtained from patients with femoral head necrosis, and 16 were collected from osteoarthritic cartilage. The thermogravimetrical analysis was performed with the use of a MOM Derivatograph (MOM, Budapest, Hungary), and the TG, DTG and DTA curves were determined.
Results: It was found, that the total water content of intact (healthy) cartilage was 80,79% (SD: 7.09%), of necrotic femoral head was 87.80% (SD: 8.06%), of the osteoarthritic samples was 86.71% (SD: 7.84%). To remove the cartilage extra cellular water content 52.33 (SD: 6.68) kJ/M energy was needed in normal samples, in aseptic femoral head necrosis needed 70.25 kJ/M